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Effects of Sublethal Exposure to Zinc Chloride on the Reproduction of the Water Flea, *Moina irrasa* (Cladocera)

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As a result of industrial activities, aquatic ecosystems have been contaminated increasingly by metals. Such occurrences pose a threat to aquatic organisms in particular and to the whole ecosystem in general. Because of their importance as part of the food chains in the freshwater ecosystem, as well as their high vulnerability to metal contaminants, the cladoceran crustaceans have attracted attention by toxicologists. The acute toxicity of metals to cladocerans has been well documented by Hall *et al.* (1986), Chandini (1988), and Zou and Bu (1994). However, most of the investigations in chronic toxicity deal with metals not essential for life such as mercury and cadmium (Wong and Wong 1990; Chandini 1991; Chen and McNaught 1992). It is well known that exposure to mercury and cadmium can generally render inhibitory effects. However, the effects of sublethal exposure of cladocerans to metals such as zinc and selenium, which are essential for life at trace level, are not as well known. As one of the major metal contaminants in freshwater ecosystems, zinc is of ecotoxicological interest. The effects of sublethal zinc exposure, especially at low concentrations, on the reproduction of cladocerans are poorly understood. The objective of this study was to investigate the effects of exposure to a wide range of sublethal concentrations of zinc chloride on the reproduction of *Moina irrasa*, a cladoceran commonly found in the freshwaters of the Yangtze delta of China.

MATERIALS AND METHODS

Moina irrasa used in the experiments were from a stock culture cloned from a parthenogenic female at a temperature of 20°C, pH 7.0 ± 0.2 and a food concentration of 2 ~ 3 x 10^4 yeast cells/mL. The stock culture was maintained in a 20 L aquarium. The neonates (age < 24 hr) used in the tests were obtained by isolating pregnant adults from the stock culture a day earlier. The chronic tests were conducted at four $ZnCl_2$ concentrations plus controls each with 20 replicates. $ZnCl_2$ concentrations ranged from 25 to 200 µg $ZnCl_2$/L, roughly corresponding to one eighth to one hundred percent of the 48-hr LC_{50} of $ZnCl_2$ at 20°C and pH 8.0 (Zou and Bu 1994). All experiments were done on neonates. One neonate was introduced into each test vessel (10 mL test tube) filled with 5 mL test medium containing a food

Pressent address: Department of Ecology, Evolution, and Organismal Biology, Tulane University, New Orleans, LA 70118 concentration of 2 ~ 3 × 10^4 yeast cells/mL. The test media were prepared by adding aliquots of $ZnCl_2$ stock solution and yeast to distilled water. The selected water quality variables at 25°C were as follows: conductivity 0.5 ~ 2.0 µS/cm, hardness less than 5 mg/L as $CaCO_3$ and dissolved oxygen 6.2 ~ 6.5 mg/L. The test media were renewed every three days. All the experiments were performed at 20°C and pH 7.0 ± 0.2.

About six days after the initiation of tests, *M. irrasa* started to reproduce and produced broods every one to three days. Reproduction was recorded daily. Offspring were removed with a pipette. Most of the experimental *M. irrasa* died by the end of the third week. All survivors perished within the next three days without reproducing. The endpoints observed included size of individual brood, brood number, mean brood size, progeny of the first two and three broods, and total number of neonates reproduced during their life time. Multiple comparison of group means (Tu 1985) was used to determine the NOEC value and the specific concentrations of toxicants that gave significantly different results from the control. The EC_{50} value with 95% confidence intervals was calculated according to Gad and Weil (1984).

RESULTS AND DISCUSSION

Table 1 summarizes the effects of Zn exposure on the reproduction of *M. irrasa.* The effects of Zn on the individual brood size of *M. irrasa* were either stimulatory or inhibitory depending on the strength and the duration of exposure. At 25 µg $ZnCl_2$/L, Zn significantly increased the size of the first and second broods ($P < 0.05$, Table 1) but the size of the fifth brood was significantly reduced ($P < 0.01$, Table 1). At higher concentrations the stimulatory effects of Zn gradually diminished while the inhibitory effects became more pronounced (Table 1). For instance, Zn exposure at 50 µg $ZnCl_2$/L still had the tendency, though not statistically significant, to increase the size of the first two broods and started to initiate inhibitory effects on the fourth brood, while at 200 µg $ZnCl_2$/L the effects were all inhibitory (Table 1). The inhibitory effects of Zn were also manifested by the significant reduction of brood number at all concentrations (Table 1). The disappearance of beneficial effects may have been due to excessive accumulation of Zn in *M. irrasa.*

To study the proper parameter for measuring the chronic toxicity of Zn, a series of endpoints were screened. The progenies of the first two and three broods were not included in the calculations owing to stimulatory effects of Zn at low concentrations. The total progeny in the entire population of adults appeared to be the best suited for the study. The NOEC for total progeny was 25 µg $ZnCl_2$/L and the EC_{50} obtained for the total progeny was 249.8 µg $ZnCl_2$/L with the 95% confidence interval ranging from 689.8 to 90.24 µg $ZnCl_2$/L.

The effects of exposure to metals at sublethal concentrations on the reproduction of cladocerans are not well documented. A life table study by Wong (1993) showed that exposure to Zn at concentrations from 0.01 to 0.70 mg/L caused a reduction in the life table parameters of *Moina macrocopa,* such as net reproductive rate and intrinsic rate of population increase. However, comparison of data obtained by Wong (1993) and

Table 1. Effects of zinc exposure to *Moina irrasa* on the number of progeny produced in each brood.

$ZnCl_2$ (μg/L)	First Brood	Second Brood	Third Brood	Fourth Brood	Fifth Brood	Sixth Brood
control	12.70±2.60 20†	20.05±4.22 20	15.50±4.05 20	11.71±3.72 17	16.08±4.01 13	13.30±3.33 10
25.0	15.95±5.08* 20	24.00±6.15* 18	18.94±7.58 16	11.67±5.41 15	9.00±3.92** 7	5.00±1.73 3
50.0	12.82±4.35 17	21.06±4.04 17	12.73±7.24 11	6.50±3.27** 6	—	—
100.0	11.00±3.54 19	18.47±4.03 17	11.69±4.98* 16	6.80±3.22** 10	4.13±2.17*** 8	—
200.0	10.11±4.23* 19	15.94±3.46** 18	14.36±4.70 14	5.55±2.25*** 11	6.00±2.83 2	—

$ZnCl_2$ (μg/L)	First Two Broods	First Three Broods	Brood Number	Mean Brood Size	Total Progeny
control	32.85±5.18 20	48.10±7.90 20	5.25±1.52 20	15.30±2.16 20	78.20±18.33 20
25.0	39.83±9.29** 18	58.81±6.86*** 16	4.00±1.59* 20	17.03±3.55 20	65.90±22.00 20
50.0	33.88±4.62 17	46.55±7.03 11	3.06±0.97*** 17	15.41±2.76 17	45.60±11.80*** 17
100.0	29.65±4.85 17	41.25±5.29** 16	3.74±1.45** 19	11.65±2.25*** 19	42.80±15.20*** 19
200.0	26.06±4.33*** 18	39.14±5.84** 14	3.42±1.17*** 19	12.10±2.56*** 19	39.80±11.30*** 19

† Number of data sets.
* Significantly different from control at $P < 0.05$.
** Significantly different from control at $P < 0.01$.
*** Significantly different from control at $P < 0.001$.

the present study can not be made as Wong (1993) did not study the individual brood basis and it is not known how the size of each brood was affected at low Zn concentrations. Owsley and McCauley (1986) reported that exposure to selenite-selenium at 0.1 ~ 0.4 mg/L significantly reduced the total number of neonates of the first three broods produced by F1 *Ceriodaphnia affinis*. However, these authors did not discuss why the progenies of the first three broods produced by F2 *C. affinis* exposed at 0.05 and 0.1 mg/L selenite-selenium were almost 45% and 40% larger than that of control. This may suggest that Se exposure at low concentrations was beneficial for the reproduction of the F2 *C. affinis*.

It is not known why Zn was beneficial for the reproduction of *M. irrasa* at low concentrations. Perhaps Zn at low concentrations is able to stimulate the feeding of *M. irrasa* hence leading to increase in the brood size. Zinc is known to be involved in the endocrine processes and reproduction of mammals (Prasad 1993) and it is possible that a certain degree of Zn accumulation may be conducive to the reproduction of *M. irrasa*. These conclusions, however, require further supportive data.

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